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Efficacy of Lantibiotic Treatment of *Staphylococcus aureus*-Induced Skin Infections, Monitored by *In Vivo* Bioluminescent Imaging

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Staphylococcus aureus **is a bacterial pathogen responsible for the majority of skin and soft tissue infections. Antibiotics are losing their efficacy as treatment for skin and soft tissue infections as a result of increased resistance in a variety of pathogens, including *S. aureus*. It is thus imperative to explore alternative antimicrobial treatments to ensure future treatment options for skin and soft tissue infections. A select few lantibiotics, a group of natural defense peptides produced by bacteria, inhibit the growth of numerous clinical *S. aureus* isolates, including methicillin-resistant strains. In this study, the antimicrobial activities of nisin, clausin, and amyloliquecidin, separately administered, were compared to that of a mupirocin-based ointment, which is commonly used as treatment for *S. aureus*-induced skin infections. Full-thickness excisional wounds, generated on the dorsal surfaces of mice, were infected with a bioluminescent strain of *S. aureus* (strain Xen 36). The infections were monitored in real time using *in vivo* bioluminescent imaging. Lantibiotic treatments significantly reduced the bioluminescence of *S. aureus* Xen 36 to a level similar to that recorded with mupirocin treatment. Wound closure, however, was more pronounced during lantibiotic treatment. Lantibiotics thus have the potential to be used as an alternative treatment option for *S. aureus*-induced skin infections.**

Skin and soft tissue infections (SSTIs) are common bacterial infections, and increased antimicrobial resistance limits the options available for treatment of SSTIs (1, 2). Mupirocin (Bactroban)-based ointments, one of the recommended treatments for *Staphylococcus aureus*-induced SSTIs, are losing their effectiveness, especially against antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA) (3, 4). Increased resistance to vancomycin and linezolid, considered "drugs of last resort" for severe MRSA and vancomycin-resistant *S. aureus* infections, respectively, has also been reported (5–8). This further limits treatment options for *S. aureus*-induced SSTIs, especially those caused by antibiotic-resistant strains, and alternatives are desperately needed to ensure future treatment efficacy. As a viable alternative, researchers have focused on antibiotics that either target cell wall synthesis or destabilize the cell membrane (9). Certain conserved cellular components involved in cell wall biosynthesis cannot be altered or replaced by simple mutations without having a detrimental effect on the bacteria, and this makes them valuable targets (9). Lipid II is an essential precursor in the formation of bacterial cell walls and is an example of a viable alternative target for next-generation antibiotics. Lantibiotics are small cationic antimicrobial peptides (cAMPs) produced by several Gram-positive bacteria that disrupt cell wall biosynthesis by binding to lipid II (10). Furthermore, certain lantibiotics, in addition to inhibiting cell wall biosynthesis, can form pores in the bacterial cell membrane, resulting in leakage of intracellular material (10). Several lantibiotics are active against antibiotic-resistant pathogens, and their efficacy in treating bacterial infections has been reported in several animal models (11–14).

Wound healing is as important as treating infection and is a complex process that involves a number of highly programmed phases (15, 16). These phases are regulated by the immune system, which in turn can be negatively influenced by a variety of factors, including stress, diabetes, obesity, and nutrition (15, 17). Antimicrobial peptides, such as cathelicidin LL-37 and defensins, play an important role in immunity by acting as antimicrobials and/or immunomodulatory molecules to resolve infection and speed up the recovery process (18). Lantibiotics are also able to modulate the innate immune system, with nisin showing promising immunomodulatory activity (19, 20). The immune response triggered by nisin is able to protect the host against infection caused by Gram-positive and Gram-negative bacteria. This response is unexpected, as nisin displays antimicrobial activity toward Gram-negative bacteria only when combined with a chelating agent or when the outer membrane has been damaged (20, 21). In an unrelated study, Heunis and coworkers (14) observed accelerated wound healing when *S. aureus*-induced skin infections were treated with nisin incorporated into nanofibers. These studies imply that nisin, and possibly other lantibiotics, may have immunomodulatory activity that can be exploited to boost the immune system to combat infection and enhance wound healing.

Here, we report on the efficacy of the lantibiotics nisin, clausin, and amyloliquecidin (AmyA), a novel two-component lantibiotic produced by *Bacillus amyloliquefaciens*, in treating *S. aureus*-in-

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duced skin infections. Importantly, the effect on wound healing and closure was investigated to evaluate the efficacy of these lantibiotics as novel wound repair and regeneration agents.

MATERIALS AND METHODS

Growth media were from Biolab Diagnostics (Gauteng, South Africa) unless otherwise stated. Polyvinyl alcohol (PVA) (87 to 89% hydrolyzed; M_w, 146,000 to 186,000) and trifluoroacetic acid (TFA) were from Sigma-Aldrich (St. Louis, MO). The Pierce bicinchoninic acid (BCA) protein assay was from Thermo Scientific (Rockford, IL). Gauze and micropore surgical tape were from AlphaPharm (Stellenbosch, South Africa). Biopsy punches were supplied by Stellenbosch Medical Supplies (Stellenbosch, South Africa). Isoflurane was from Safe Saline Pharmaceuticals (Isofor; Gauteng, South Africa), and buprenorphine was from Schering-Plough Ltd. (Tamgesic; Cape Town, South Africa).

Preparation of lantibiotics. Lantibiotics were purified and antimicrobial activity was tested as discussed in the supplemental material. Freeze-dried high-performance liquid chromatography (HPLC)-purified samples were reconstituted in 0.1% (vol/vol) TFA for antimicrobial assays and phosphate-buffered saline (PBS) (pH 7.4) for animal trials. Peptide concentrations were determined using the BCA protein assay according to the manufacturer's instructions. Lantibiotics were prepared to a final concentration of 50 μM in 0.1% (vol/vol) TFA for antimicrobial assays and 250 μM in PBS (pH 7.4) containing 2.5% (wt/vol) PVA for animal trials. The lantibiotic suspensions were stored at 4°C throughout each animal trial. In the case of AmyA, the α- and β-peptides were combined in a 1:1 molar ratio. The suspensions were freshly prepared before each trial.

Animals used. Ethical clearance to conduct research on animals was granted by the ethics committee of Stellenbosch University (SU-ACUM14-00009). Adult female nude mice (weighing 20 to 30 g) were used for infection studies and housed in separate cages under controlled environmental conditions (12-h light/dark cycles; 20 to 22°C). The animals were fed sterile standard rodent feed and water. Closure of noninfected wounds was investigated in male nude mice (weighing 20 to 30 g) housed under similar conditions. Wound infection studies were conducted in three independent trials, and studies of the closure of noninfected wounds were conducted in two independent trials.

Full-thickness wound generation and infection with *S. aureus* Xen 36. A full-thickness excisional wound was made on the dorsal surface of each mouse by using a 6-mm biopsy punch. The mice received buprenorphine (~0.03 mg/kg of body weight) subcutaneously as an analgesic before wound generation and for the first 3 days post-wound generation. A single *S. aureus* Xen 36 colony was used to inoculate brain heart infusion (BHI) broth supplemented with kanamycin (200 μg/ml) and was incubated overnight at 37°C. The overnight culture was subinoculated into fresh medium and grown to an optical density at 600 nm (OD_{600}) of 1.0 to 1.2 (~2×10^8 CFU/ml). Cell counts were verified by serial dilution and plating onto BHI agar supplemented with kanamycin (200 μg/ml). Bacteria were harvested (10,000 × *g*; 2 min), washed twice with sterile PBS (pH 7.4), and resuspended in sterile PBS (pH 7.4) to the original OD_{600}. The wounds were each inoculated with *S. aureus* Xen 36 (2×10^6 CFU/wound), left to dry for 5 min, and then covered with Parafilm and gauze. The dressings were kept in place with micropore surgical tape.

Treatment and evaluation of *S. aureus* Xen 36 wound infections. Mice ($n = 9$ in each treatment group) were left for 3 h postinfection before treatment commenced. Wounds were treated with 12.5 μl (250 μM) of AmyA, clausin, or nisin applied directly onto the wound and dispersed evenly over the surface. The same volume (12.5 μl) of mupirocin ointment (GlaxoSmithKline, Research Triangle Park, NC) was also dispensed onto wounds using a micrometer syringe attached to a Leur fitting. The wounds of control mice were treated with 2.5% PVA in PBS (pH 7.4).

The first set of bioluminescent images, recorded using an *in vivo* imaging system (IVIS 100; Caliper Life Sciences, Perkin-Elmer, Hopkinton, MA), was 5 min after treatment. Follow-up treatments with the same dose of lantibiotics, mupirocin, and control suspension were 2, 4, and 6 days after infection. Bioluminescent images were recorded daily for 7 days and analyzed using Living Image software (v3.0) from Caliper Life Sciences. Bioluminescence was measured in a region of interest (ROI) (25 by 25 pixels) and expressed as $\log_{10}$ photons per second per square centimeter per steradian ($ps^{-1}\ cm^{-2}\ sr^{-1}$). All images were taken with the dressings removed. On day 7, the mice were euthanized with an overdose of pentabarbitone sodium (Euthapent; Kyron Laboratories Ltd., Benrose, South Africa). The wounds were excised and homogenized in sterile PBS (pH 7.4), and the homogenate was serially diluted in sterile saline and plated onto BHI agar supplemented with kanamycin (200 μg/ml). The plates were incubated at 37°C for 24 h, and colonies were enumerated to determine the numbers of viable *S. aureus* Xen 36 bacteria present in the wounds.

Digital images were taken of wounds ($n = 6$ per treatment group) to determine the effect of treatment on wound closure. Digital photographs were analyzed using the software program ImageJ (NIH Research Services Branch [http://rsbweb.nih.gov/ij/]). Wound size on day n was expressed as a percentage relative to the wound size on day 0 ($D_n/D_0 \times 100$, where D_n is the wound size on day n and D_0 is the wound size on day 0).

Effect of lantibiotics on the closure of noninfected wounds. Full-thickness excisional wound generation and treatment of mice ($n = 5$ per treatment group) were as previously described. Digital images of wounds were taken, and wound closure was determined as previously described. The mice were monitored for 7 days, after which they were euthanized and the wounds were excised for histological analysis. The excised wounds were fixed in 4% formaldehyde in 0.1 M PBS (pH 6.5). Samples were processed using automated procedures to impregnate and subsequently embed the samples in paraffin wax. Five-micrometer sections were made using a rotary microtome, and the samples were stained with hematoxylin and eosin.

Statistical analysis. All the data were analyzed using GraphPad Prism (version 6.05), and statistical differences between groups were determined using two-way analysis of variance (ANOVA) and an unpaired *t* test. The statistical analyses used are indicated for each data set. A difference was considered statistically significant when the *P* value was <0.05. Errors were calculated as standard errors of the mean (SEM).

RESULTS

Lantibiotic activity against *S. aureus in vitro*. Clinical isolates of *S. aureus*, beta-hemolytic streptococci, *Enterococcus* spp., and *Listeria* spp. were used as targets to determine the antimicrobial spectrum of AmyA, clausin, and nisin (see Tables S1 and S2 in the supplemental material). All three lantibiotics were active against *S. aureus* (including MRSA strains), beta-hemolytic streptococci, and *Listeria* spp. Fewer species were inhibited by AmyA, and concentrations higher than those of clausin and nisin (based on MIC values) were required to have the same antibacterial effect against *S. aureus* Xen strains (see Table S2 in the supplemental material). Preliminary MIC values for mupirocin (from mupirocin ointment) against *S. aureus* Xen 36 (the strain used for *in vivo* studies) were less than 5 μM, and the concentration used on mice was 46.1 mM mupirocin (in 12.5 μl mupirocin ointment).

Efficacy of lantibiotics in the treatment of *S. aureus*-induced wound infections. Full-thickness excisional wounds on the dorsal surfaces of mice, generated with a biopsy punch (6-mm diameter), were infected with 2×10^6 CFU of the bioluminescent *S. aureus* strain Xen 36. The progression of infection was evaluated daily for 7 days by measuring bacterial bioluminescence (IVIS; Perkin-Elmer, Waltham, MA, USA). Antimicrobial and control treatments were applied to the wounds 3 h after infection, followed by additional applications on days 2, 4, and 6 (Fig. 1).

All antimicrobial treatments reduced the bacterial load, as indicated by a reduced bioluminescent signal emitted from *S. aureus*

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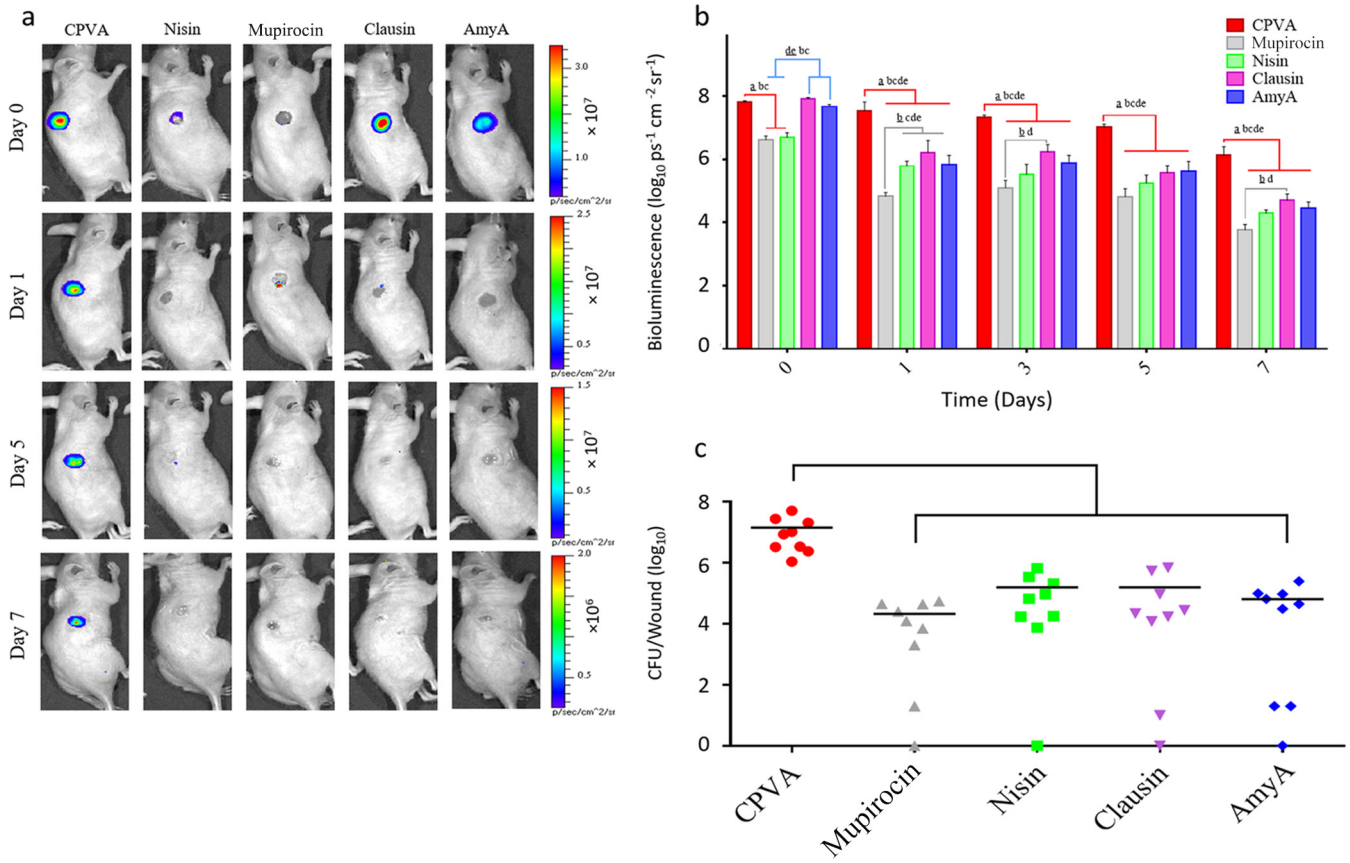

FIG 1 *In vivo* efficacy of antimicrobials in the treatment of *S. aureus*-induced skin infection in mice ($n = 9$ per treatment group). (a) Representative images of *in vivo S. aureus* bioluminescence following antimicrobial treatment. (b) Dynamics of *in vivo S. aureus* bioluminescence after antimicrobial treatment. The letters and brackets above the bars (means and SEM) indicate groups with statistical differences as determined by two-way ANOVA. (c) Viable *S. aureus* Xen 36 cells enumerated from excised wounds on day 7. The horizontal lines and brackets represent means and statistical differences, respectively. Statistical analysis was performed using unpaired *t* tests.

Xen 36 (Fig. 1a and b). Wounds treated with the control polyvinyl alcohol (CPVA) solution had stable bioluminescence throughout the 7-day trial period. Treatment with mupirocin (the antibacterial standard for comparison) and nisin resulted in an almost immediate reduction in bioluminescence, i.e., within 5 min of treatment. Significant reductions in bioluminescence readings were recorded 1 day after treatment with AmyA and clausin. Treatment with mupirocin resulted in the lowest bioluminescence readings throughout the trial period (Fig. 1b).

Wounds were excised on day 7, and the number of viable cells of *S. aureus* Xen 36 present in the tissue was determined (Fig. 1c). Viable-cell numbers of *S. aureus* Xen 36 were significantly and similarly reduced when wounds were treated with lantibiotics or mupirocin compared to CPVA treatment. Despite recorded differences in bioluminescence, there were no significant differences in the numbers of *S. aureus* Xen 36 cells isolated from wound tissue treated with any of the antimicrobials. Tissue samples with the highest viable-cell numbers included wounds treated with CPVA (5.1×10^7 cells; mean, 1.4×10^7 cells per wound), followed by nisin (6.6×10^5 cells; mean, 1.6×10^5 cells per wound) and clausin (7.0×10^5 cells; mean, 1.6×10^5 cells per wound).

Effects of antimicrobial treatments on wound closure. Wound healing can be hampered by infection, and it would thus be ideal if an antimicrobial agent could also facilitate wound healing. Wound sizes were therefore measured and compared to those of CPVA-treated wounds to determine the effects of the lantibiotics on closure in infected wounds (Fig. 2a). All the treatments resulted in a gradual decrease in wound size, with CPVA, mupirocin, nisin, clausin, and AmyA treatments resulting in 54.8% ± 4.6%, 52.7% ± 6.9%, 63.9% ± 4.7%, 66.7% ± 1.6%, and 69.3% ± 0.7% closure, respectively, after 7 days. All the treatments, including CPVA treatment, resulted in smaller wounds on day 7 than treatment with mupirocin. Clausin- and AmyA-treated wounds were smaller than CPVA-treated wounds. Although not significant, nisin-treated wounds were also smaller than CPVA-treated wounds.

The effect of antimicrobial treatment on wound closure in the absence of infection was also studied (Fig. 2b). Noninfected wounds treated with CPVA, mupirocin, nisin, clausin, and AmyA resulted in wound closure slightly less than that observed for infected wounds (48.7% ± 3.53%, 35.7% ± 3.04%, 55.2% ± 3.01%, 60.1% ± 2.20%, and 55.5% ± 4.78%, respectively). However, the difference was not significant, with the exception of AmyA and clausin on days 5 and 7 (see Fig. S3 in the supplemental material). Mupirocin treatment of uninfected wounds resulted in delayed wound closure; this is in agreement with the closure of infected wounds treated with mupirocin. Clausin treatment had the most drastic effect on wound closure, reducing wound size by ~11.4%

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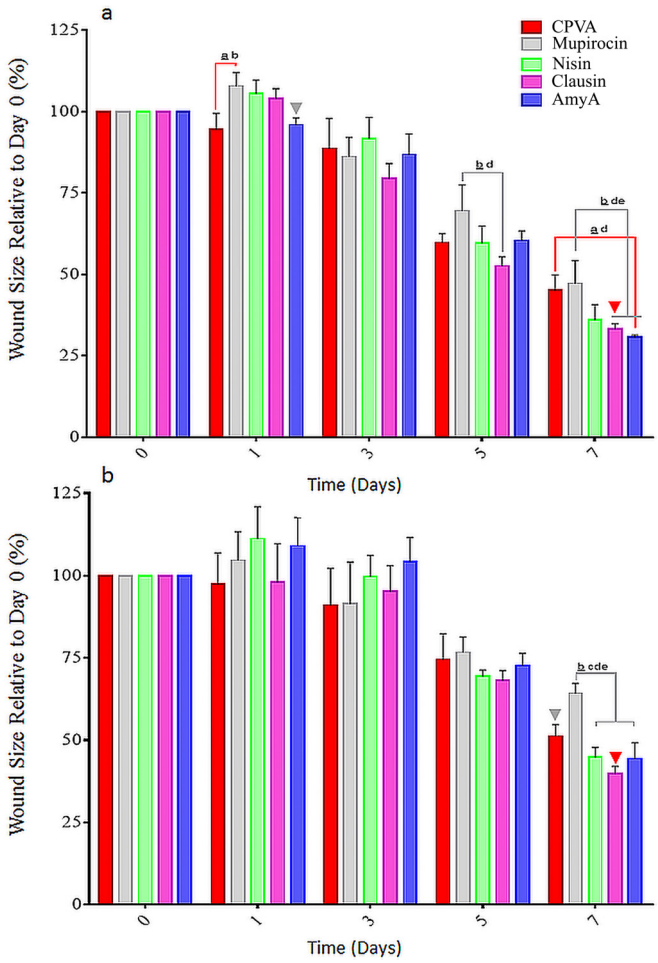

FIG 2 Effects of antimicrobial treatment on closure of infected and noninfected wounds. The letters and brackets above the bars (means and SEM) indicate groups with statistical differences as determined by two-way ANOVA. (a) Closure of wounds infected with *S. aureus* Xen 36 ($n = 6$ per treatment group). The gray arrowhead indicates a statistical difference between mupirocin- and AmyA-treated wounds, and the red arrowhead indicates a statistical difference between CPVA- and clausin-treated wounds, as determined by unpaired *t* tests. (b) Closure of noninfected wounds ($n = 5$ per treatment group). The gray arrowhead indicates a statistical difference between mupirocin- and CPVA-treated wounds, and the red arrowhead indicates a statistical difference between CPVA- and clausin-treated wounds, as determined by unpaired *t* tests.

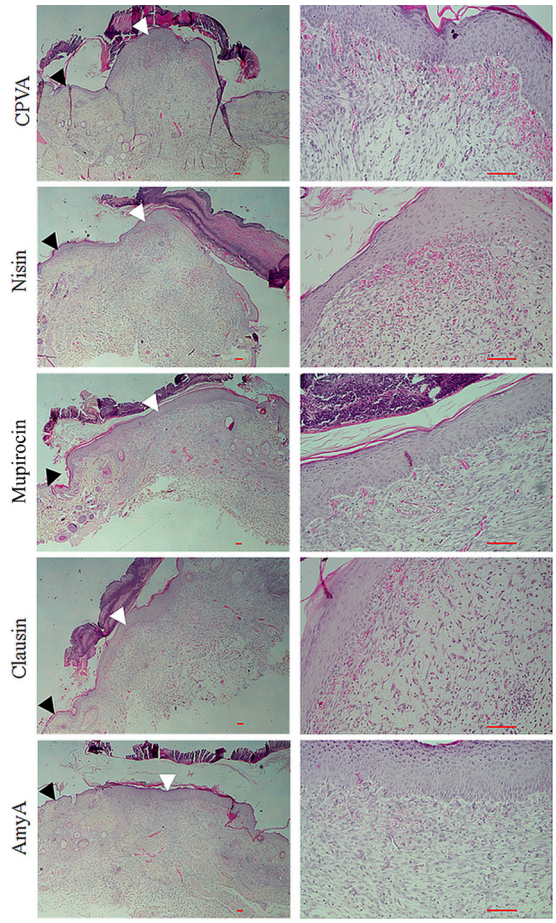

FIG 3 Representative photomicrographs of sections from noninfected wounds excised on day 7, stained with hematoxylin and eosin. The black and white arrowheads indicate undamaged and damaged tissue, respectively. Scale bars, 50 μm. The images on the left and right were taken at ×20 and ×40 (within the damaged area) magnification, respectively.

more than CPVA-treated wounds and ~24.4% more than mupirocin-treated wounds. Although the difference was not significant, AmyA- and nisin-treated wounds were also smaller than CPVA-treated wounds on day 7.

Histological analysis revealed differences in neutrophil infiltration, neovascularization, and epithelialization among the different groups (Fig. 3). Excised CPVA-treated wounds displayed significant epithelialization on day 7, as evidenced by the relatively thick epithelial layer in the wound area compared to healthy, undamaged skin in the same sample. Widespread neovascularization was visible, with damage to the skin ultrastructure evident at higher magnification. The significant degree of edema and neutrophil infiltration suggests that recovery was in the early inflammatory phase. In contrast, in mupirocin-treated wounds, the degree of vascularization was not as pronounced, with the increase in epithelial layer thickness in the wound area much less than that observed in CPVA-treated wounds. Mupirocin-treated wounds showed signs of neovascularization. However, the neovascularization response was less pronounced than that observed in CPVA-treated wounds. The decrease in epithelialization and the delayed neovascularization associated with mupirocin treatment suggest that the recovery process may have been delayed, which is supported by the delayed wound closure. All the lantibiotic-treated groups were associated with significant epithelialization and neovascularization. Interestingly, these treatments showed less vascularization than CPVA-treated wounds, while the significant epithelialization in these groups argues against a delayed recovery process. Also, compared to the CPVA-treated group, lantibiotic-treated groups exhibited relatively few infiltrated neutrophils in recovering tissue, suggesting that recovery had already progressed further than in the CPVA-treated controls.

DISCUSSION

The skin is the largest organ in the body and acts as a barrier protecting the host from the outside environment. The microbiota naturally present on the skin lives in symbiosis with the host (22). Disruption of the skin barrier can lead to dysbiosis, which in

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turn leads to microbial invasion by commensal and noncommensal bacteria. This may result in severe SSTIs and can affect wound healing (22, 23). This study investigated the efficacy of the lantibiotics nisin, clausin, and the newly described AmyA in the treatment of *S. aureus*-induced skin infections in mice (24). The results were compared with those obtained using a commercially available mupirocin-containing ointment. All the lantibiotics used in the current study were as effective as mupirocin in reducing the bacterial loads of *S. aureus*-infected wounds. Importantly, the lantibiotic treatments did not negatively influence wound healing, as was observed after mupirocin treatment. Wound healing and the severity of infection can be affected by several factors, including the route of infection and the immunological response. The route of infection plays a role in clinical severity, with intradermal and superficial infections resulting in different inflammatory responses (4). Athymic nude mice do not display the same clinical severity of response to intradermal *S. aureus* infection as wild-type (BALB/c) mice, with clinical severity proposed to be driven by the inflammatory response to bacteria rather than the bacterial burden (25). However, in the current study, clinical severity measured by the rate of wound closure in superficial *S. aureus*-induced skin infections in athymic mice was similar to that reported for superficial *S. aureus* skin infection studies in wild-type (C57BL/6 or BALB/c) mice (4, 14).

Lantibiotics are classified into distinct classes based on their modification machinery and further subdivided into different groups based on amino acid sequence (26, 27). The three lantibiotics used in this study are from different classes. Nisin belongs to the nisin-like lantibiotics and clausin to the epidermin-like lantibiotics. Both are classified as class I lantibiotics. Amyloliquecidin is a two-component class II lantibiotic, with the individual peptides classified as mersacidin-like (α-peptide) and LtnA2-like (β-peptide) lantibiotics (24). Nisin is the prototypical lantibiotic and is active against several Gram-positive bacterial species, including antibiotic-resistant strains, and is effective in the treatment of microbial infections (13, 14, 28–30). Clausin has not been studied to the same extent, with only a few studies investigating its activity and mode of action (31, 32). Amyloliquecidin is a newly isolated lantibiotic, and its antimicrobial activity has not been reported. It has to be pointed out that, based on amino acid sequence alignments, AmyA is different from other two-component lantibiotics, as well as amylolysin, produced by *B. amyloliquefaciens* (see Fig. S2 in the supplemental material) (24, 33, 34). Most lantibiotics target the pyrophosphate moiety of lipid II and subsequently inhibit cell wall biosynthesis (35, 36). Certain lantibiotics form pores in the cell membrane after lipid II binding by forming a membrane-spanning complex (10, 35). Several lantibiotics are active against antibiotic-resistant pathogens, and their efficacy in treating bacterial infections has been reported in several animal models (11–14, 29, 30). However, resistance to lantibiotics has been described in the literature, and the majority of the mechanisms responsible for resistance involve alterations in the charge and permeability of the cell wall or membrane, respectively (37). Resistance mechanisms include alteration of the cell wall and membrane, such as increases in the positive charge of the cell wall or changes in the phospholipid composition of the cell membrane (37–46). Other resistance mechanisms include biofilms, spore formation, and, in some cases, specific antilantibiotic mechanisms (37). The development of resistance to lantibiotics, specifically alteration of lipid II, may be reduced due to the unique binding of lantibiotics to the pyrophosphate moiety, which is essential for lipid II function and structure (9, 36). Additionally, the dual mode of action of some lantibiotics poses a significant challenge to target organisms and may help limit the onset of resistance. These characteristics make lantibiotics ideal candidates for next-generation antimicrobials.

Little has been published on the *in vivo* treatment of topical infections using lantibiotics. However, lantibiotics are effective in the treatment of *S. aureus* infections when administered via the subcutaneous, intraperitoneal, intranasal, and intravenous routes (11, 12, 29, 30, 47, 48). Nisin incorporated into nanofibers could be used to treat topical *S. aureus* infections (14). Nisin-eluting nanofibers significantly reduced the bacterial load, as shown by bioluminescence and viable-cell counts, similar to the results of the current study. These findings are promising, taking into account the fact that several lantibiotics, including those in the current study, are active against antibiotic-resistant strains (11, 12, 28, 47).

The immediate reduction in bioluminescence when wounds were treated with nisin or mupirocin may be due to their modes of action (Fig. 2). Nisin inhibits cell wall biosynthesis by binding to the cell wall precursor lipid II and disrupting peptidoglycan synthesis. Once bound, nisin forms pores in the cell membrane, followed by leakage of cellular contents (10, 13, 35). Mupirocin blocks protein synthesis through inhibition of isoleucyl-tRNA synthetase and is bactericidal, or bacteriostatic at low concentrations (49–51). The rapid decrease in bioluminescence recorded when wounds were treated with nisin could be due to rapid cell lysis, whereas the decrease in readings recorded with mupirocin treatment could be due to a bactericidal, rather than a bacteriolytic, action. Irrespective of the mode of activity, cells of *S. aureus* Xen 36 would not be able to emit a detectable bioluminescent signal. Clausin and AmyA treatment did not show an initial decrease in bioluminescence, but there are plausible reasons for this. Clausin is an epidermin-like lantibiotic, and they are based on amino acid sequences shorter than those of the nisin-like lantibiotics. Although both epidermin-like and nisin-like lantibiotics can effectively bind to lipid II, pore formation by epidermin-like lantibiotics is affected by membrane thickness (10). This may explain the delayed reduction in bioluminescence from cells treated with clausin. Second, two-component lantibiotics, such as AmyA, require two peptides to act synergistically to induce cellular leakage. The α-peptide binds to lipid II, followed by binding of the β-peptide to the α-peptide–lipid II complex. This interaction then results in pore formation (52). Thus, in an *in vivo* situation, it can be expected that the reaction may take place at a reduced rate and could therefore account for AmyA not being able to rapidly reduce bioluminescence. It is also possible that one or both of the AmyA peptides interact with lipids/membranes other than those found in the target organism and, by doing so, delay antimicrobial activity.

From this study, it is evident that lantibiotics, in addition to controlling infection, do not negatively influence wound healing compared to CPVA and mupirocin treatments, with similar effects observed for wound closure in both infected and noninfected wounds. In contrast, despite the effectiveness of mupirocin in reducing the bacterial burden, it delayed wound closure compared to all the other treatments. The observation in the mupirocin-treated wounds of less epithelialization, as well as the delayed neovascularization, suggests that the recovery process may have been delayed (53). Similar cases of delayed wound closure have been

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reported for mupirocin formulations (54). These facts add to the concern raised by an actual delayed wound closure rate associated with mupirocin, which highlights the necessity to find more suitable alternatives, such as the ones reported in the current study. The composition of the drug delivery vehicle can also influence antimicrobial effectiveness and wound healing (4, 55). Interestingly, there were no significant differences in wound closure between infected and noninfected wounds treated with CPVA. Kim and coworkers (56) reported similar results while studying the dynamics of neutrophil infiltration during full-thickness wound healing in mice. In their study, mice were intraperitoneally injected with granulocyte-macrophage colony-stimulating factor (GM-CSF) (wounds not infected) or saline (wounds not infected) or, alternatively, not injected (wounds infected with *S. aureus*). The authors found that, despite an increase in neutrophil recruitment resulting from infection with *S. aureus* or treatment with GM-CSF, wound closure rates in all groups were similar to that of the saline-injected control. In our study, the lantibiotics did not negatively influence wound closure, with all wounds being smaller than CPVA- and mupirocin-treated wounds (Fig. 2). These results, along with those from the histological analysis, may indicate an alternative or additional mechanism by which lantibiotics facilitate regeneration of damaged tissue. Similar results were obtained when wounds were treated with nisin-eluting nanofibers, including earlier signs of epithelialization and lack of neutrophil infiltration (14), suggesting faster resolution of inflammation and more efficient tissue repair. Lantibiotics are able to induce chemokines involved in wound healing. Interleukin 8, growth-related oncogene α, and monocyte chemoattractant protein 1 are induced by nisin and gallidermin, with nisin treatment resulting in stronger induction of these chemokines (20). Nisin and gallidermin are classified into different groups, namely, nisin-like and epidermin-like lantibiotics, respectively. The lantibiotics in the current study were also from different groups, and although not statistically significant, differences in the effects of these lantibiotics were evident. This indicates that structural differences in lantibiotics possibly influence their modulation of the immune system.

In conclusion, we have demonstrated the effectiveness of lantibiotics in the treatment of *S. aureus*-induced skin infections, as well as their effects on the closure of infected and noninfected wounds. Of particular interest is the fact that the current study is the first to investigate and report on the antimicrobial properties of the newly identified two-component lantibiotic amyloliquecidin. From the data reported here, it is evident that the lantibiotics assessed are as effective in reducing the bacterial load on wounds as mupirocin treatment, which is one of the recommended treatments for *S. aureus*-induced skin infections. Furthermore, treatment with lantibiotics is equivalent to or better than treatment with mupirocin, especially with respect to wound closure rates. The specific role(s) of these promising lantibiotics in wound healing and how their structural properties might influence this interaction are areas that require further investigation. However, the efficacy in terms of both antimicrobial and wound-healing properties of the lantibiotics used in this study, compared to mupirocin, effectively illustrates the potential of lantibiotics in the treatment of skin infections.

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